IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Douglas A. RUSSELL *et al.*
Appl. No.: 09/824,200
Filed: April 3, 2001
For: ***Expression and Purification of Bioactive, Authentic Polypeptides from Plants***

Art Unit: 1637
Examiner: Jeffrey N. FREDMAN
Atty. Docket: 18337.006
Conf. No.: 5401

AMENDMENT AND RESPONSE

Attn: BOX AF

Commissioner for Patents
Washington, D.C. 20231

Sir:

In reply to the Final Office Action mailed January 13, 2003 (Paper No. 12), Applicants submit the following amendment and remarks.

IN THE CLAIMS:

Please **add** new claim 94 as follows:

94. (New) The method of claim 1, wherein said plant host system is a maize host system.

Table 3–1 Approximate Chemical Compositions of a Typical Bacterium and a Typical Mammalian Cell

Component	Percent of Total Cell Weight	
	E. Coli *Bacterium*	*Mammalian Cell*
H_2O	70	70
Inorganic ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, etc.)	1	1
Miscellaneous small metabolites	3	3
Proteins	15	18
RNA	6	1.1
DNA	1	0.25
Phospholipids	2	3
Other lipids	—	2
Polysaccharides	2	2
Total cell volume:	2×10^{-12} cm^3	4×10^{-9} cm^3
Relative cell volume:	1	2000

Proteins, polysaccharides, DNA, and RNA are macromolecules. Lipids are not generally classed as macromolecules even though they share some of their features; for example, most are synthesized as linear polymers of a smaller molecule (the acetyl group on acetyl CoA) and self-assemble into larger structures (membranes). Note that water and protein comprise most of the mass of both mammalian and bacterial cells.

The Specific Interactions of a Macromolecule Depend on Weak Noncovalent Bonds[2]

A macromolecular chain is held together by *covalent* bonds, which are strong enough to preserve the sequence of subunits for long periods of time. Although the sequence of subunits determines the information content of a macromolecule, utilizing that information depends largely on much weaker, *noncovalent* bonds. These weak bonds form between different parts of the same macromolecule and between different macromolecules. They therefore play a major part in determining both the three-dimensional structure of macromolecular chains and how these structures interact with one another.

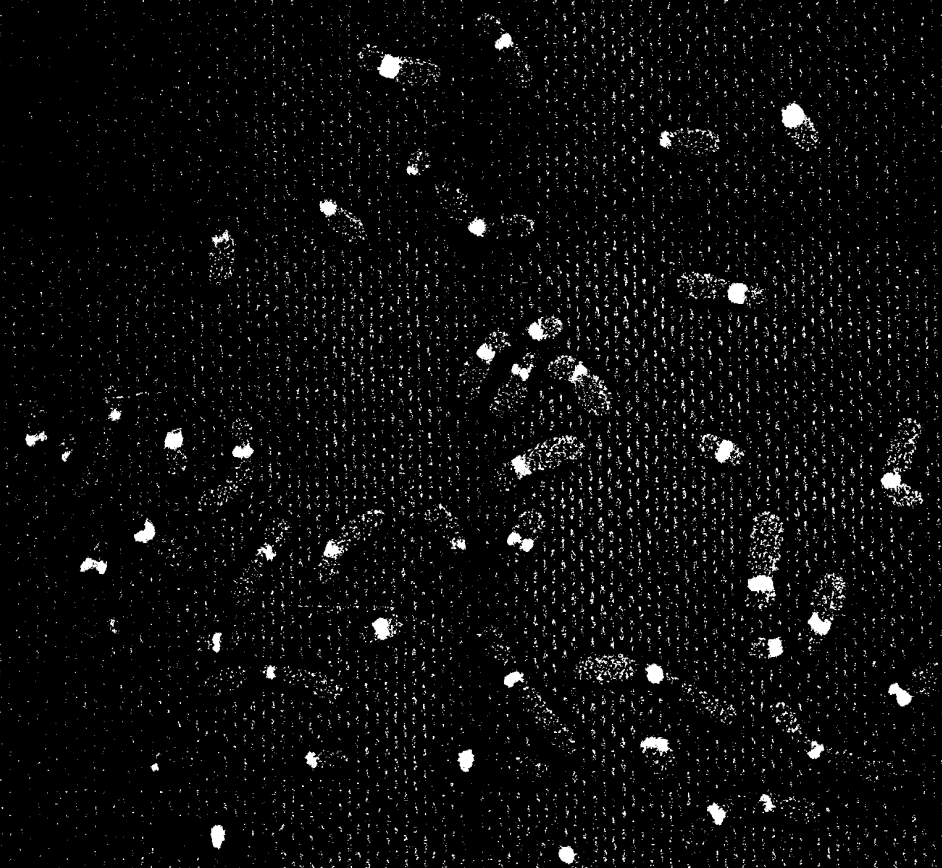
The noncovalent bonds encountered in biological molecules are usually classified into three types: **ionic bonds, hydrogen bonds,** and **van der Waals attractions.** Another important weak force is created by the three-dimensional structure of water, which tends to force hydrophobic groups together in order to minimize their disruptive effect on the hydrogen-bonded network of water molecules (see Panel 2–1, pp. 46–47). This expulsion from the aqueous solution generates what is sometimes thought of as a fourth kind of weak noncovalent bond. These four types of weak bonds are the subject of Panel 3–1, pp. 90–91.

In an aqueous environment each noncovalent bond is 30 to 300 times weaker than the typical covalent bonds that hold biological molecules together (Table 3–2) and only slightly stronger than the average energy of thermal collisions at 37°C. A single noncovalent bond—unlike a single covalent bond—is therefore too weak to withstand the thermal motions that tend to pull molecules apart, and large numbers of noncovalent bonds are needed to hold two molecular surfaces together. Large numbers of noncovalent bonds can form between two surfaces only when large numbers of atoms on the surfaces are precisely matched to each other (Figure 3–2), which accounts for the specificity of biological recognition, such as occurs between an enzyme and its substrates.

The weak noncovalent forces determine how different regions of the same macromolecule fit together, in addition to determining how that macromolecule will interact with other molecules. However, as explained at the top of Panel 3–1, atoms behave almost as if they were hard spheres with a definite radius (their

MOLECULAR BIOLOGY OF THE CELL

SECOND EDITION



Bruce Alberts · Dennis Bray · Julian Lewis
Martin Raff · Keith Roberts · James D. Watson

Biotechnol. Prog. 1992, 8, 285-290

ARTICLES

Effect of Oxygen Supply on the Suspension Culture of Genetically Modified Tobacco Cells

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The effect of oxygen supply on the cultivation of the genetically modified tobacco cells and the formation of a foreign protein, β-glucuronidase (GUS), was investigated in 250-mL Erlenmeyer flasks, a 5-L stirred tank fermenter, and a 7-L air-lift fermenter. The oxygen supply was varied by using different volumes of medium in the case of the 250-mL Erlenmeyer flask culture or by the different aeration rate in the case of the two types of fermenters tested. Higher oxygen supply stimulated cell growth and increased oxygen consumption rate, the level of phenolics, and GUS productions.

Introduction

Large-scale plant suspension cultures can be employed for the production of pharmaceuticals, flavors, food colors, etc. However, the slow growth rate and the low product yield of plant cells are the two major obstacles for their active commercial utilization. These limitations can be partially overcome by selecting fast growing and high yielding cell lines, by optimizing growth media, or by improving fermenter design.

More drastic improvements may be possible by employing recombinant DNA technology to modify the metabolic pathways to overproduce a desired natural metabolite or to insert foreign genes to produce a protein product. However, the modification of metabolic pathways may be very difficult due to the complex nature of metabolic pathways involved in the metabolite production. On the other hand, current plant biotechnology made it possible to insert foreign genes into plants or plant cells by agrobacterium-mediated transformation techniques and those transgenic plants or cells could produce foreign proteins such as chloramphenicol acetyltransferase (Gorman et al., 1982; An, 1986), neomycin phosphotransferase (Reiss et al., 1984), β-glucuronidase (Jefferson et al., 1987), human serum albumin (Sijmons et al., 1990), antibodies (Hiatt et al., 1989; Düring et al., 1990; Hein et al., 1991), and proteinase inhibitor (Johnson, et al., 1989).

In an effort to test the feasibility of the large-scale production of foreign proteins in plant cell cultures, we have demonstrated the production of a model bacterial protein, chloramphenicol acetyltransferase (CAT), from genetically modified tobacco cells by using various culture environments including shake flasks, a stirred fermenter, and a bubble column in batch, semibatch, and continuous modes (Hogue et al., 1990). We have also studied the stability of β-glucuronidase (GUS) production from genetically modified tobacco cells by monitoring GUS activity during the 295 days (composed of 42 sub-batch cultures) of 10 independently transformed cell lines. We found that 8 cell lines out of 10 cell lines either maintained or increased the stability of the production during the entire testing period, which indicated that the stability of transferred foreign gene in plant cells was very good (Gao et al., 1991).

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Oxygen supply is known to affect the cell growth and the secondary metabolite production (Snape et al., 1989; Tate and Payne, 1991; Leckie et al., 1991; Kobayashi et al., 1989; Kato et al., 1975; Tanaka, 1981). Generally, growth rate increases with the increas of oxygen supply until it reaches a maximum (Snape et al., 1989; Tate and Payne, 1991; Leckie et al., 1991; Kobayashi et al., 1989). However, further increase of the oxygen supply by increasing the aeration rate or the stirring speed can decrease the growth rate not because of the oxygen effect but because of the increased hydrodynamic shear (Tanaka, 1981) or carbon dioxide stripping (Hegarty et al., 1986). The increase of oxygen supply can increase the production of secondary metabolites (Tate and Payne, 1991; Kobayashi et al., 1989) or can alter the pattern of product accumulation during a batch cultivation (Leckie et al., 1991). However, there is no report concerning the effect of oxygen on genetically modified plant cells.

In this paper, we report the effect of oxygen supply on cell growth, GUS production, and secondary metabolite (phenolics) formation in suspension cultures of genetically modified plant cells by employing different culture environments such as shake flask, a stirred tank fermenter (STF), and an air-lift fermenter (ALF).

Materials and Methods

Plant Cell Line and a Model Foreign Protein Product. The plant cell line used in this study was tobacco cells, *Nicotiana tabacum* 1 (NT-1), which were genetically modified to carry a foreign gene, β-glucuronidase (GUS) from bacteria (Gao et al., 1991). The tobacco cell line was chosen because of its excellent characteristics as a suspension culture in view of its growth rate and stability. The GUS gene was chosen because its assay is sensitive and reliable.

Culture Medium. Genetically modified plant cells were grown in a liquid medium containing 4.3 mg/mL Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.18 mg/mL KH_2PO_4, 0.1 mg/mL inositol, 1 μg/mL thiamine hydrochloride, and 0.2 μg/mL 2,4-dichlorophenoxyacetic acid (2,4-D) as growth hormone. Kanamycin (42 μg/mL) was added in order to control the bacterial contamination.

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Batch Cultures. The genetically modified tobacco cells were cultivated in 250-mL Erlenmeyer flasks, a 5-L (working volume of 3 L) stirred tank fermenter (STF), and a 7-L (working volume of 4.5 L) air-lift fermenter (ALF), all in a batch mode. The culture was maintained at 30 °C in the dark. The inoculum size for all batch cultivation was 5%.

The amount of oxygen supply in the flask was manipulated by cultivating cells in three different medium volumes, 30, 90, and 150 mL, in the same size 250-mL Erlenmeyer flasks, which were shaken on a gyratory shaker (Model G25, New Brunswick Scientific Co.) at 150 rpm. The STF (New Brunswick Scientific, Model F5) was agitated with a flat-bladed impeller (5.6 cm in diameter, four blades) with the rotation speed of 150 rpm. The STF was aerated through a one-hole nozzle with various aeration rates. The ALF was constructed by placing a glass draft tube (5 cm in outer diameter, 0.175 cm thick, and 47.8 cm long) centrally in an outer glass column (11.4 cm in outer diameter, 0.7 cm thick, 89 cm long). The outer column was fastened by two plates. The top plate has ports for sampling, gas exhaust, and medium addition. The bottom plate has ports for aeration, steam sterilization, and draining. The draft tube was placed 3 cm above the bottom plate. Sterile air was passed through a 36-hole (0.1 cm in diameter) ring sparger (7.4 cm in diameter) which was located 5 cm above the bottom plate (Gao, 1990).

Cell and Phenolics Concentrations. The cell and phenolics concentrations of suspension culture were determined as previously described (Hooker et al., 1989).

Sucrose, Glucose, and Fructose Assays. Sucrose concentration in the culture suspensions was determined by the anthrone method (Handel, 1968). Glucose concentration was determined by using the hexokinase enzymatic assay kit (Boehringer Mannheim, No. 139041), and fructose concentration was measured by using the same method as that for glucose after the sample was treated with an enzyme, phosphoglucose isomerase (Boehringer Mannheim, No. 127396).

Protein and GUS Assay. About 1 g of cell suspension was centrifuged for 1 min and was resuspended in plant extraction buffer (17% sucrose, 0.1% ascorbic acid, 0.1% cysteine hydrochloride, and 0.1 M Tris-HCl, pH 8.0). It was sonicated and centrifuged to obtain crude protein extract. Protein concentration of the extract was measured by the Bradford Reagent (Bio-Rad Laboratories, Richmond, CA). GUS activity was measured by the colorimetric method (Jefferson, et al., 1986). One unit of GUS enzyme represents 1 nmol of *p*-nitrophenol formed at 37 °C in 1 min.

Determination of k_La and Oxygen Consumption Rate. The overall volumetric oxygen transfer coefficient (k_La) was determined by the transient gassing-out technique (Lee, 1992; Van't Riet, 1979) using a dissolved oxygen meter (YSI MODEL 58, Yellow Springs Instrument Co.). The oxygen consumption rate of the culture was measured by the dynamic technique (Lee, 1992; Taguchi and Humphrey, 1966).

Results and Discussion

GUS Cell Cultures in Shake Flasks. In order to study the effect of oxygen supply on the growth and metabolic production in genetically modified plant cells, we varied the medium volume in the 250-mL Erlenmeyer flasks from 30 to 150 mL. The increase of the medium volume in the flask decreases the liquid surface area but increases the oxygen demand due to the increased liquid volume and cells. Furthermore, the increase of the medium also decreases the mixing intensity in the flask due to the decrease of the free space in the flasks for liquid movement during agitation.

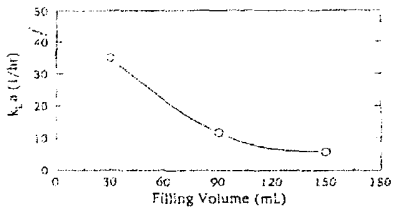

Figure 1. Effect of filling volume (water) in a 250-mL Erlenmeyer flask on volumetric oxygen transfer coefficients k_La.

Figure 1 shows the change of the volumetric oxygen transfer coefficient (k_La) as a function of the volume of water in a 250-mL Erlenmeyer flask placed on a shaker at 150 rpm. As expected, k_La was decreased as the filling volume was increased. The range of k_La values was 6–35 h^{-1}, which showed that a wide range of oxygen supply can be manipulated by varying the medium volume in shake flask.

Figure 2 shows the changes of dry and wet cell concentrations during 8 days of batch cultivation of the genetically modified tobacco cells in 250-mL Erlenmeyer flasks with three different medium volumes (30, 90, and 150 mL). Each culture had about 2 days of lag phase, followed by the exponential stage. The specific growth rates (dry weight basis) during the experimental period between 2 and 5 days were 0.66, 0.52, and 0.43 day^{-1} for the 30-, 90-, and 150-mL cultures, respectively. The decrease of the filling volume increased not only the specific growth rate but also the maximum cell concentration. The growth condition for the 150-mL culture was so poor that the stationary phase could not be reached within 8 days.

Figure 3 shows the rate of oxygen consumption by the GUS cells during the first four days of the 30- and 150-mL batch cultures. Initially, both cultures had the same oxygen uptake rate. As the cultures started to grow exponentially after 2 days, the 30-mL culture exhibited much higher oxygen consumption rate per unit volume than the 150-mL culture did, which might be attributed to the more active growth in the 30-mL flask. However, the comparison of the specific oxygen uptake per unit of dry cell mass (the lower part of Figure 3) also showed that the cells in 30-mL culture consumed more oxygen [the average of 0.359 mmol/(h·g)] than that in 150-mL culture [the average of 0.207 mmol/(h·g)]. These results indicated that fast growing cells had more active respirations than slow growing cells.

Figure 4 shows the total protein and GUS activity of the cultures in shake flasks. The increase of the oxygen supply increased not only the total productivity of protein, but also the specific GUS activity per unit amount of protein produced. The decreases of GUS production and GUS specific activity in the cultures with 90- and 150-mL were mainly caused by poor oxygen supply and insufficient mixing in these cultures. The total protein of the cultures followed the same trends as the cell mass concentration of the cultures, implying that the protein content inside the cell was fairly constant. It is also interesting to note that the specific GUS activity for the 30-mL run reached the maximum value after 6 days that corresponds to the second day of stationary phase, and it declined slightly thereafter. This implies that the GUS production is growth related.

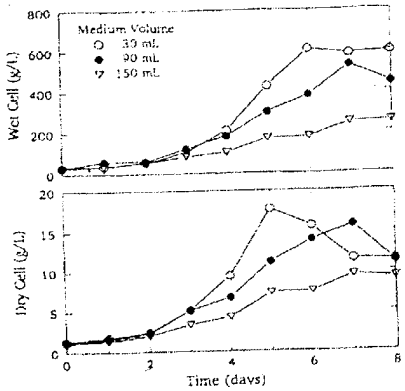

Figure 2. Effect of medium volume in a 250-mL Erlenmeyer flask on the growth of the genetically modified tobacco cell cultures.

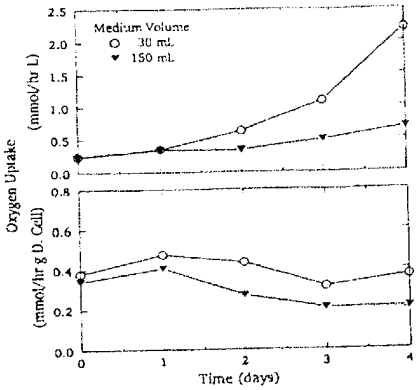

Figure 3. Effects of medium volume and cell ages in a 250-mL Erlenmeyer flask on the specific oxygen uptake rate (in millimoles per hour per liter) and the overall oxygen uptake rate (in millimoles per hour per gram) by the genetically modified tobacco cell cultures.

The hydrolysis of sucrose and the utilization of glucose and fructose were monitored during the batch cultures and are shown in Figure 5. Before sucrose can be utilized by plant cells, it is hydrolyzed into glucose and fructose. The concentration of sucrose in the 30-mL culture declined quickly and dropped almost to zero after five days, showing a high metabolic activity for sucrose conversion in the culture. The conversion rates of sucrose (on the basis of first five days) in 30-, 90-, and 150-mL cultures were 5.6, 3.5, and 3.1 g/day, respectively. The sucrose conversion was faster than the consumption rates of glucose and fructose, resulting in the increases of glucose and fructose concentrations in all three cultures during the exponential stage. The rates of glucose consumption in the 30-, 90-, and 150-mL cultures (on the basis of first five days) were 2.8, 1.7, and 0.8 g/day, respectively, showing the increased consumption rate with the increase of oxygen supply. The rates of fructose consumption were about half of those of glucose (1.4, 1.0, and 0.4 g/day for the 30-, 90-, and 150-mL cultures, respectively) during the first five days, and the accumulated fructose was consumed after glucose was depleted.

The secondary metabolites, total phenolics, were also monitored during the batch cultures, and the results are

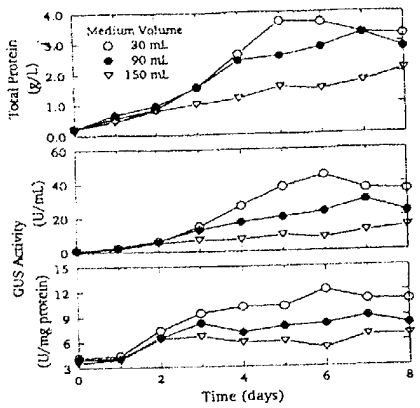

Figure 4. Effect of medium volume in a 250-mL Erlenmeyer flask on the total protein concentration (in grams per liter), total GUS activity (in units per milliliter), and specific GUS activity (in units per milligram) of the genetically modified tobacco cell cultures.

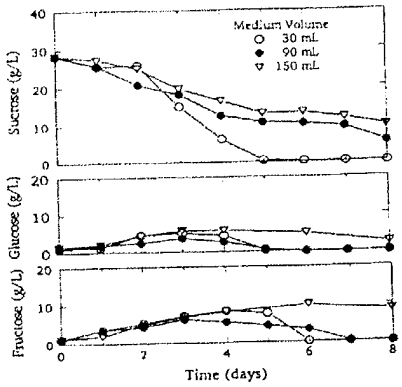

Figure 5. Effect of medium volume in a 250-mL Erlenmeyer flask on the carbohydrate utilization patterns of the genetically modified tobacco cell cultures.

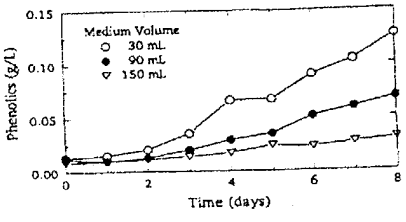

Figure 6. Effect of medium volume in a 250-mL Erlenmeyer flask on the phenolics production in genetically modified tobacco cell cultures.

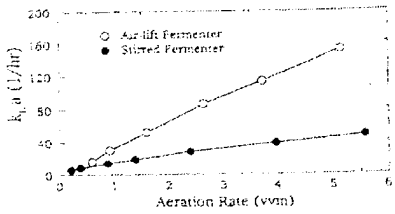

Figure 7. Effect of aeration rate on volumetric oxygen transfer coefficients $k_L a$ in a 5-L STF and in a 7-L ALF.

shown in Figure 6. As the oxygen supply increased by decreasing culture volume, phenolics production increased. The culture with 30-mL medium had a phenolics production of 0.126 g/L after 8 days which was 2 times and 4 times higher than those cultures with 90- and 150-mL media, respectively. This result is in agreement with some studies of secondary metabolite productions such as berberine and serpentine (Kobayashi et al., 1989; Scragg et al., 1987). As discussed previously, higher oxygen supply could stimulate the growth and metabolic activities in plant cell cultures, and therefore enhanced the metabolic pathways for phenolics production.

GUS Cell Cultures in STF and ALF. In order to investigate the effect of oxygen supply in different fermenter environments, we cultivated the genetically modified tobacco cells in a 5-L stirred tank fermenter (STF) and a 7-L air-lift fermenter (ALF) with various levels of aeration. The oxygen transfer coefficients $k_L a$ at different aeration rates in vvm (volume of air per unit volume of liquid per minute) are shown in Figure 7. The $k_L a$ increased almost linearly with the aeration rate in STF (agitation speed, 150 rpm). Even though the $k_L a$ value was increased with the increase of aeration rate up to 48 h^{-1} at 5.6 vvm, the operation of STF with the aeration rate exceeding 1.1 vvm was impractical for the plant cell cultivation, due to excessive foam and cell aggregates forming in the head space of the fermenter. The rate of increase in $k_L a$ with the aeration rate in ALF was higher than that in STF, as also shown in Figure 7. The tall column in ALF made the gas–liquid contact more efficient by a longer residence time of rising bubbles. The internal circulation through the draft tube further increased the residence time as the small bubbles entrained through the draft tube. This showed that the ALF had a higher oxygen-supplying ability compared with STF. However, as the case in STF, overaeration in ALF caused serious foaming, which made it impractical to aerate at more than 3.5 vvm.

The batch cultures of GUS cells in the STF with different aeration rates are shown in Figure 8. As in the case of the shake flask cultures, the increase of the aeration rate increased the growth rate. The specific growth rate for the culture with 1.1 vvm was 0.64 day^{-1} (dry weight basis) during the exponential growth period between 2 and 6 days, which was very close to the best growth rate for the shaker flask culture (0.66 day^{-1} for the 30-mL culture). The culture with 1.1 vvm reached its stationary growth stage more quickly than the other cultures with lower aeration rates.

The GUS production and specific activity in STF are shown in Figure 9. The highest aeration produced the highest level of GUS per unit volume. However, at the lower aeration rates, it was difficult to find the trend due to the data scatter, though the lowest aeration (0.1 vvm) resulted in the lowest GUS concentration. The specific GUS activities (units per milligram of protein) declined

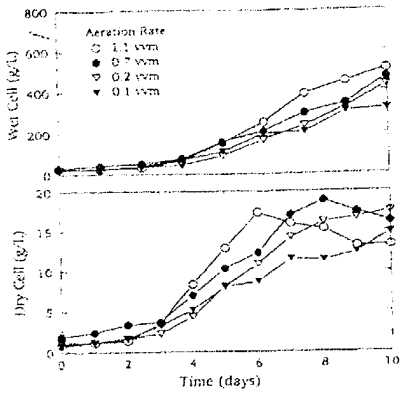

Figure 8. Effect of aeration rate on the growth of the genetically modified tobacco cell cultures in a 5-L STF.

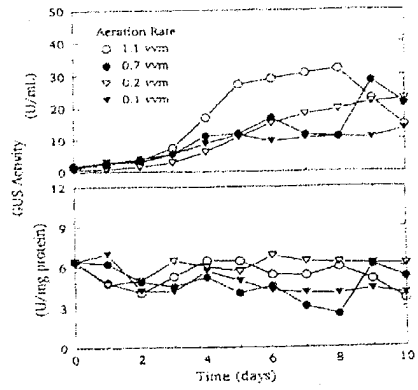

Figure 9. Effect of aeration rate on the total GUS activity (in units per milliliter) and the specific GUS activity (in units per milligram) of the genetically modified tobacco cell cultures in a 5-L STF.

for all four cases during the lag phase, followed by a slight increase during the exponential period. GUS activity became stabilized in the later exponential and stationary phases. Concerning the effect of aeration, there were also some trends that the specific activity decreased when the aeration rates were either too low (less than 0.1 vvm) or too high (larger than 0.7 vvm).

Figure 10 shows the growth curves for the batch cultures in ALF. The specific growth rate during the exponential growth period (between 2 and 5 days) for the 3.5 vvm run was 0.73 day^{-1}, which was higher than that of the best shaker flask run (0.66 day^{-1}). However, the maximum cell concentration reached in ALF was 12.8 g/L, which was lower than 17.4 g/L for the best shaker flask run (see Figure 2). The decrease of the aeration rate to 1.5 vvm did not significantly affect the specific growth rate, but the time required to reach the stationary phase took longer by 1 day. When the aeration rate was further decreased to 0.5 vvm, cells seemed to grow well in the beginning. However, as the cell population increased, the aeration was not high enough to suspend the cells which resulted

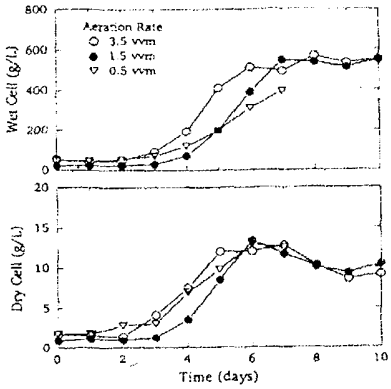

Figure 10. Effect of aeration rate on the growth of the genetically modified tobacco cell cultures in a 7-L ALF.

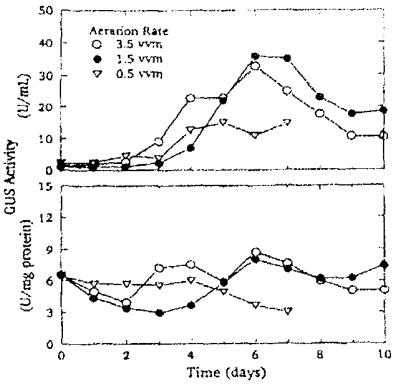

Figure 11. Effect of aeration rate on the total GUS activity (in units per milliliter) and specific GUS activity (in units per milligram) of the genetically modified tobacco cell cultures in a 7-L ALF.

in poor mixing of the medium and oxygenation. The aeration of 0.5 vvm was not high enough to create the circulation of fluid and air bubbles through the draft tube.

Figure 11 shows the GUS production and specific activity in 7-L ALF. The experimental results of GUS production followed similar trends as in the case of STF. The cultures with 3.5 and 1.5 vvm had higher GUS activity and specific activity than that in the culture with 0.5 vvm. The GUS specific activity could reach 7.6 and 7.1 units/mg for the cultures with 3.5 and 1.5 vvm, respectively. The low GUS productivity in the 0.5 vvm culture may be caused by the poor growth due to inadequate mixing in the column and possibly by the insufficient oxygen supply.

Conclusions

The effect of oxygen supply on the cultivations of the genetically modified tobacco cells and the formation of a foreign protein, β-glucuronidase, were investigated in 250-mL Erlenmeyer flasks, a 5-L STF, and a 7-L ALF. In the batch cultures in shake flasks, oxygen transfer coefficient (k_La) was controlled by various levels of medium volume in a 250-mL flask. The decrease of the medium volume provided a condition for better mixing and higher oxygen supply. The increase of the oxygen supply by decreasing the medium volume increased all of the following: specific growth rate, maximum cell concentration, oxygen uptake per unit dry cell mass, total protein production, GUS production, specific GUS activities per milligram of protein, rate of sucrose conversion, rate of glucose and fructose consumption, and secondary metabolite production (phenolics). The increase of the aeration rate in STF and ALF also showed the similar trend of increased cell growth and the GUS productions as the shaker runs.

Acknowledgment

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Recombinant Human IL-4

Catalog Number: 204-IL

Specifications and Use

Source	♦ A DNA sequence encoding the mature IL-4 protein (Yokota, T. *et al.*, 1986, Proc. Natl. Acad. Sci. USA **83**:5894) was expressed in *E. coli*.
Molecular Mass	♦ The methionyl form of the *E. coli* expressed mature human IL-4 contains 130 amino acid residues and has a molecular mass of approximately 14 kDa.
Purity	♦ > 97%, as determined by SDS-PAGE and visualized by silver stain.
Endotoxin Level	♦ < 0.1 ng per 1 µg of the cytokine as determined by the LAL method.
Activity	♦ Measured in a cell proliferation assay using a human factor-dependent cell line, TF-1 (Kitamura, T. *et al.*, 1989, J. Cell Physiol. **140**:323 - 334). ♦ The ED_{50} for this effect is typically 0.05 - 0.2 ng/mL.
Formulation	♦ Lyophilized from a 0.2 µm filtered solution in PBS containing 50 µg of bovine serum albumin per 1 µg of cytokine.
Reconstitution	♦ It is recommended that sterile phosphate-buffered saline containing at least 0.1% human serum albumin or bovine serum albumin be added to the vial to prepare a stock solution of no less than 5 µg/mL of the cytokine.
Storage	♦ Lyophilized samples are stable for greater than six months at -20° C to -70° C. ♦ Upon reconstitution, this cytokine can be stored under sterile conditions at 2° - 4° C for one month or at -20° C to -70° C for three months without detectable loss of activity. ♦ **Avoid repeated freeze-thaw cycles.**

Human Interleukin 4

Interleukin 4 is a pleiotropic cytokine produced by activated T cells, mast cells, and basophils. It was initially identified as a B cell differentiation factor (BCDF), as well as a B cell stimulatory factor (BSF1). Subsequent to the molecular cloning and expression of both human and mouse IL-4, numerous other functions have been described on B cells as well as other hematopoietic and non-hematopoietic cells, including T lymphocytes, monocytes, macrophages, mast cells, myeloid and erythroid progenitors, fibroblasts, endothelial cells, etc. IL-4 exhibits anti-tumor effects both *in vivo* and *in vitro*. Recently, IL-4 has been identified as an important regulator for the $CD4^+$ subset (Th1-like vs. Th2-like) development.

The genes for IL-4 have been mapped to human chromosome 5 and mouse chromosome 11 and are closely linked to the genes for IL-3, IL-5, IL-13 and GM-CSF. cDNAs for both murine and human IL-4 encode precursor proteins with signal peptides that are cleaved to form mature human and mouse proteins of 129 and 120 amino acid residues, respectively. Human and mouse IL-4 are species-specific in their activities.

The biological effects of IL-4 are mediated by the binding of IL-4 to specific cell surface receptors. The functional high-affinity receptor for IL-4 has now been shown to consist of a ligand binding subunit (IL-4 R) and a second subunit (β chain) that can modulate the ligand binding affinity of the receptor complex. It has been shown that in certain cell types, the gamma chain of the IL-2 receptor complex is a functional β chain of the IL-4 receptor complex.

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